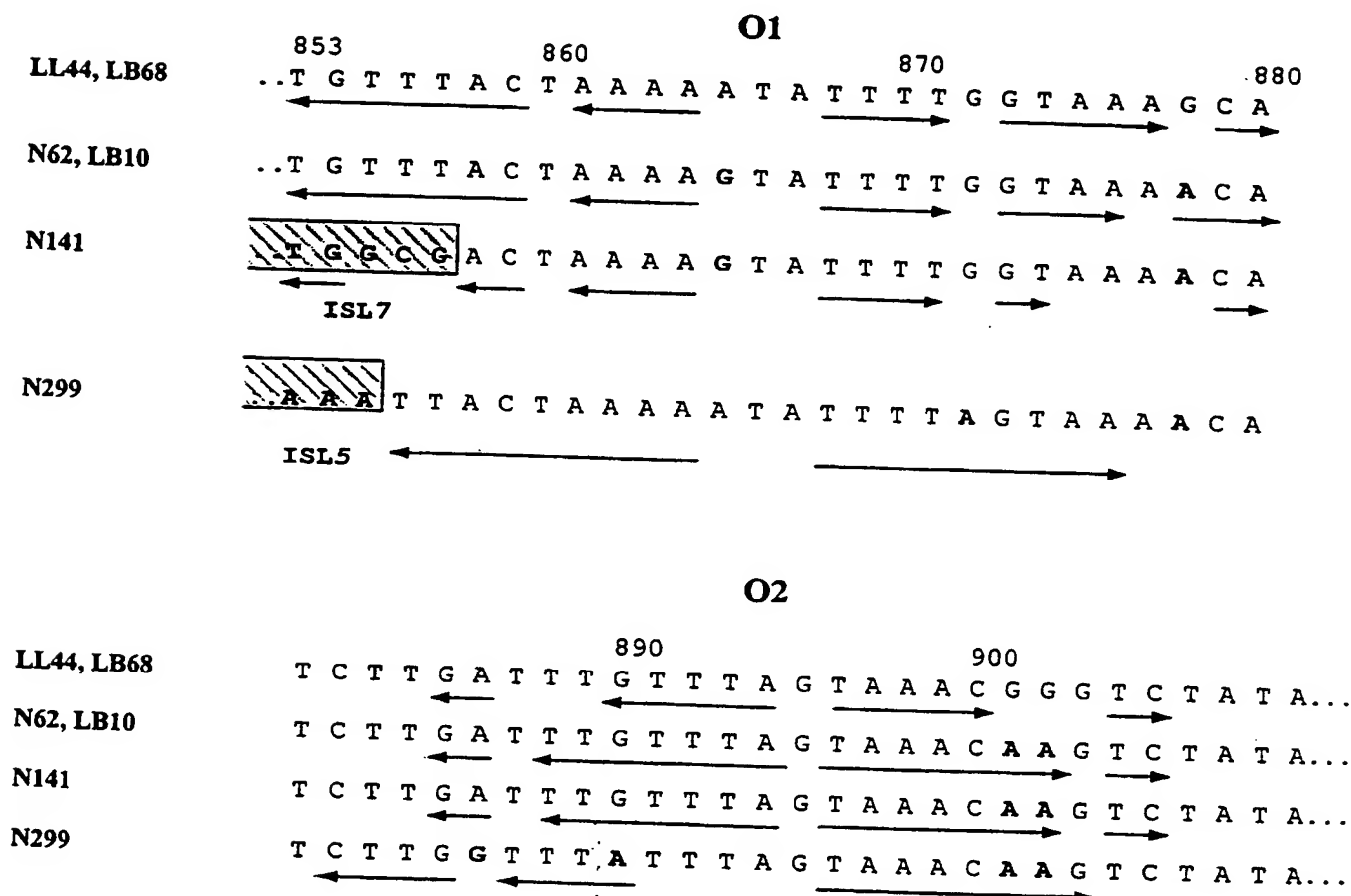


Fig. 1

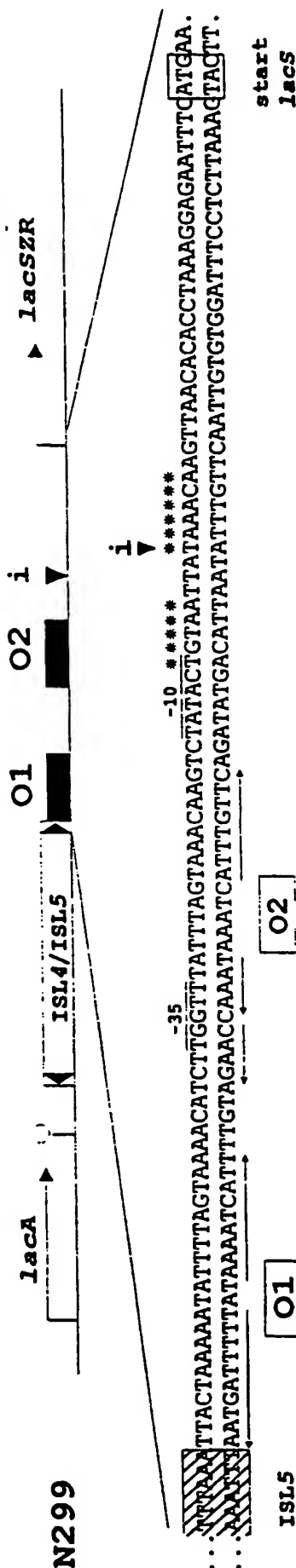


Comparison of *L. delbrueckii* operators sequences (O1 and O2). Arrows are for inverted repeats. The LL44 sequence is numbered according to figure 1. Sequence of the second helix of *lacR* (repressor) is indicated.

**Fig. 2**



**Fig. 3**



**Fig. 2 u. 3 :** Organisation of the promoter region of LL44 and N299 *lac* operon. Operators O1 and O2 are indicated by black boxes. The inverted repeats of the operators are represented by arrows. The sequence responsible for catabolite repression (CRE) is overdrawn by stars. The inverted repeat of ISL5 is boxed and shaded. The initiation of transcription is shown by an i (arrow head) (Leong-Morgenthauer et al, 1991). The promoter sequence of LL44 is numbered according to figure 1. The picture is not drawn to scale.

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1   GAATTTTGTCTGGATGCTCAGGAAGCCCGCCAGCTCAAGCTGGTGATTGAGCCACTTTTT
    stop lacZ
61  ACTGAAATATGCTACAATTGACTTAAACAGCATAAAATTTTAGTAAAAGCGAGTGAAGAAG
    RBS
121 ATGGCAACGATCAGAGAAGTGGCCAAGGCAGCCGGCGTGTGCCAGCGACGGTTTCCCGG
1   M A T I R E V A K A A G V S P A T V S R
    helix          turn          helix
181 GTCTTGAACATATGACCAGACCCTGTCCGTCAATGAGGCAACGCGGCAGAAGATATTCAA
21  V L N Y D Q T L S V N E A T R Q K I F K
241 ACTGCTGAAGCCATGCACTACCATAAGAGCCGGAAGACCAGAAAGAGCAAGCAAAAGCGC
41  T A E A M H Y H K S R K T R K S K Q K R
301 CTGGCGATCTGCCTGTGGTGTGACCAAGACCAGGAGATCAAGGACCTCTATTACTATTCA
61  L A I C L W C D Q D Q E I K D L Y Y Y S
361 ATCAGAACCAGCGCGCAAGCAGAGGCCAAGAAGCAGGGACTTGAAAGCCAGGTCAATTAT
81  I R T S A Q A E A K K Q G L E S Q V I Y
421 CCGGCTGATCCTTTGCCGATCCAGCTGCTTTAAGCGGGATTATCATGATTGGCTACCAG
101 P A D P L P D P A A L S G I I M I G Y Q
481 CAGTATTCGCCAGACCGCTTGAATGAAGTCAAAAAGTCTGGCCTGCCCTGGTCTTTGTC
121 Q Y S P D R L N E V K K S G L P L V F V
541 GATACTGACACCTTAAAATTGGGTACTGCTCAGTTGTGGCTGACTTTGGCCAGGCCATG
141 D T D T L K L G Y C S V V A D F G Q A M
601 CAGGAGGCGCTAGAGGTCTTCTGGGGGAGGGCAGGGAGCGGATCGCCCTTTTGGATGGT
161 Q E A L E V F W G Q G R E R I A L L D G
661 GATTTGGACAGTAATTTTGATAAAAAACAACCTGGTCGACTTCCGCTTCCGCGATTATAAG
181 D L D S N F D K N N L V D F R F R D Y K
    ▼
721 AAGAGCCTCGCGGCCCGCGGCCAGTACGACCCGGACTTAGTCTATGTTGGAACTTCACT
201 K S L A A R G Q Y D P D L V Y V G N F T
781 CCGCAATCTGGCTATGAAGCCATTAAAGAAGCTCTTAAGTCCGGCTCCTTCCCGAAAGCC
221 P Q S G Y E A I K E A L K S G S F P K A
841 TTGATTGCGGCTAATGACGCCATGGCTATTGGAGCATTGAAGGCCTTTAAAGAAGCTGGA
241 L I A A N D A M A I G A L K A F K E A G
901 ATTAAGTCCCAGAGGACGTCAGTCTGATTTCTTTTAAATGACACAACGGCAGCAGAATT
261 I K V P E D V S L I S F N D T T A A E F
961 GCCAACCAGCCTTGACTAGCGTACATGTAGAGACCCAGCAGATGGGCCGAGCCAGCGTC
281 A N P A L T S V H V E T Q Q M G R A S V
1021 AAGGTCATGAAAGACCTGCTGGATGATGATGAAGCCGGCACTTACAAGGTCACCTTTCCCA
301 K V M K D L L D D D E A G T Y K V T F P
1081 ACAAACCTCGTTTACCGGAATCTTGCCCAAAGCATAAGGGCATAGAGCATAATAACAG
321 T K L V Y R E S C P K A *
    ⇨
1141 CAAAGAAATAGCTTGGAGATTGATTTTCTCCAAGCTATTTTTCGTATATATTATGGCTGC
    stop asnA
1201 ATTCTGTTGATCATTCTTGGGAATGGGACAGCTTCACGAACGTGGTCCAGCTTGCAGATC
1261 CAGGCAATGACCCGTTCAAAG

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Figure 4:

Nucleotide and amino acid sequences of the *L. delbrueckii* subsp. *lactis* LL44 *lacR* gene. Start (121) and stop (1119) codons are boxed. Putative *lacR* RBS is underlined. The putative rho-independent terminator is underlined by convergent arrows. Stop codons of the beta-galactosidase (*lacZ*) and Asn t-RNA synthetase (*asnA*) genes are boxed. Insertion sequence of ISL3 is represented by a large open arrow. Single base pair deletion (722) in the mutant LZL102 is shown by an arrow head, leading to a premature stop codon (758) underlined.

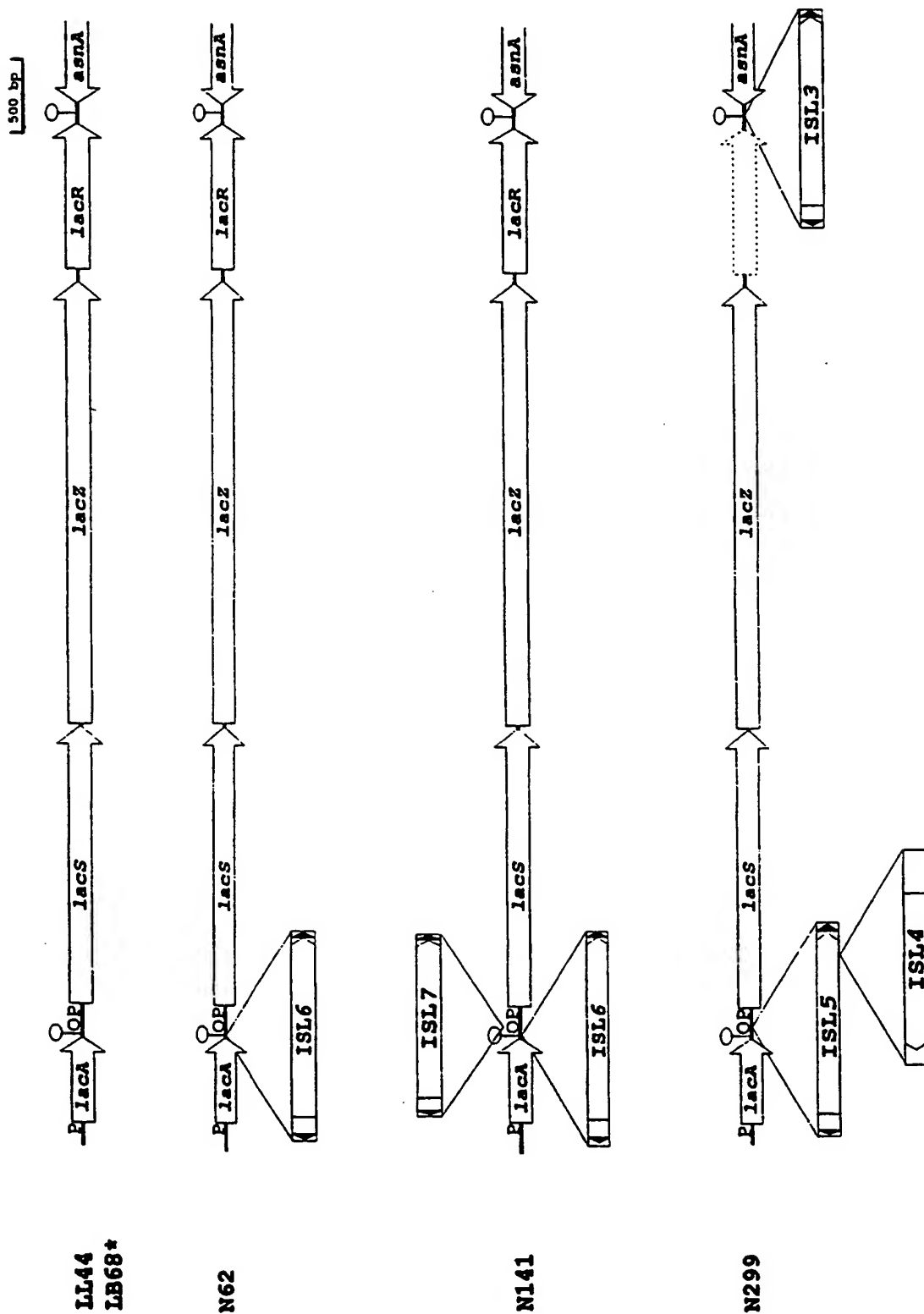
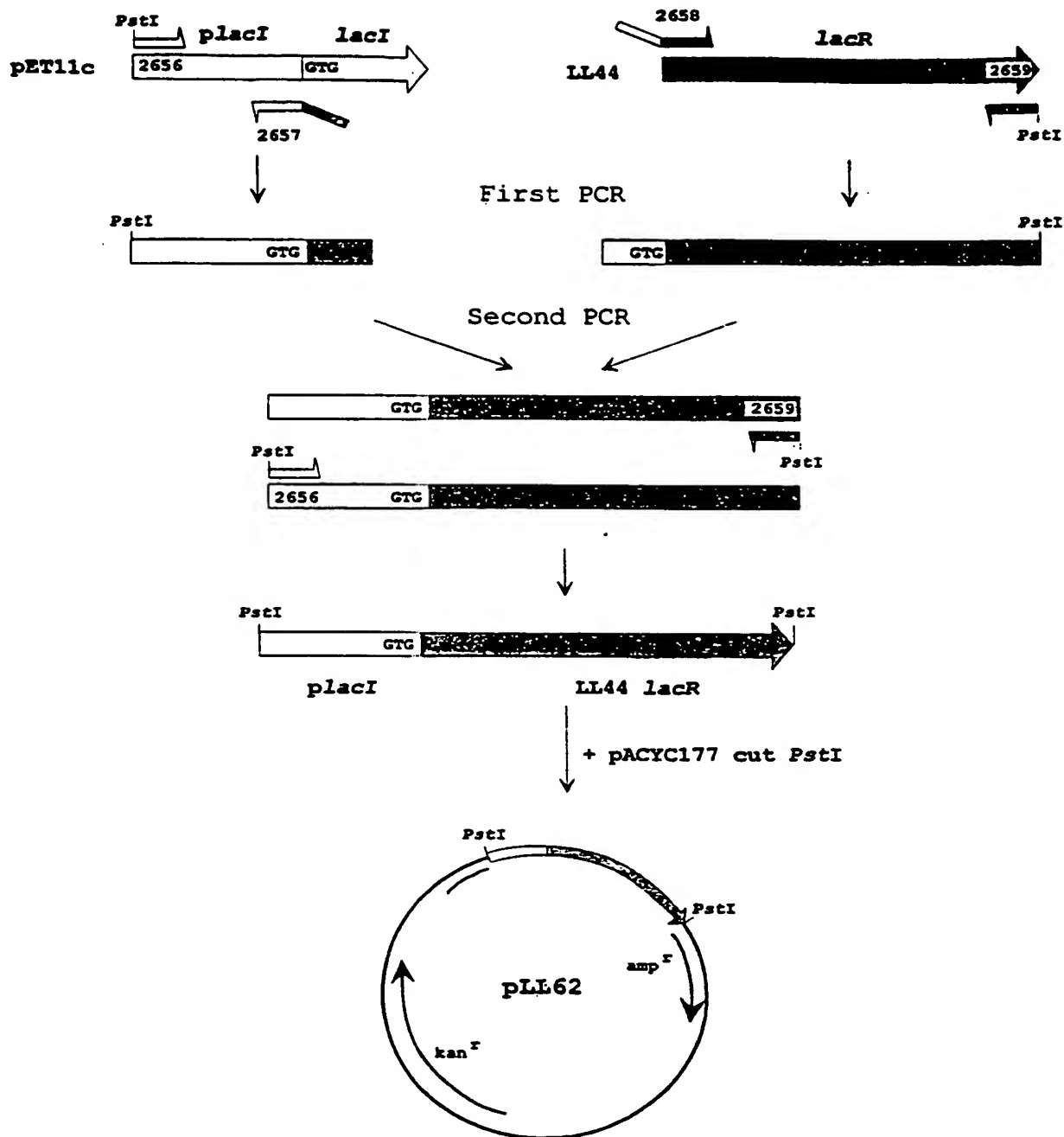
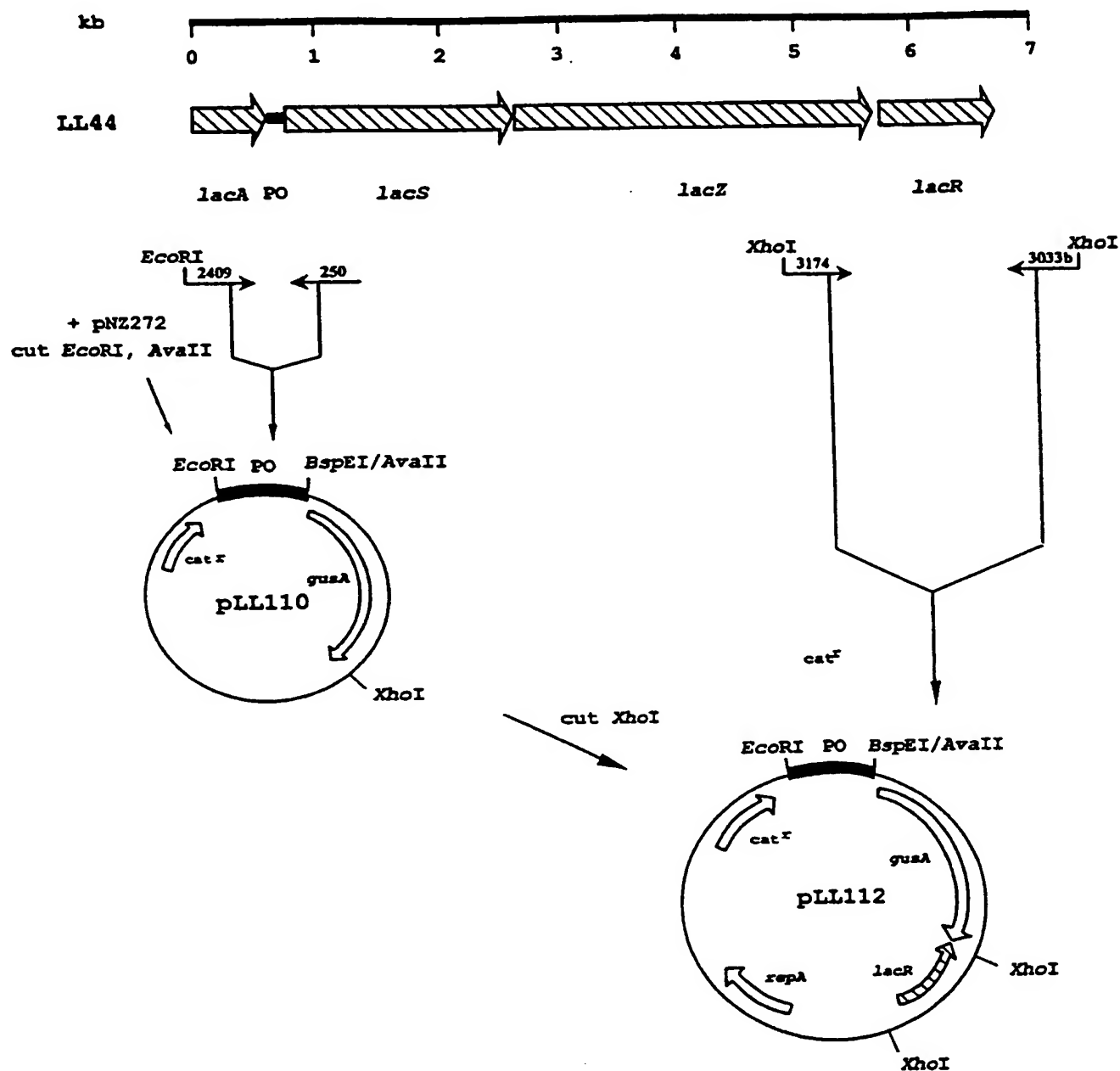


Figure 5 :

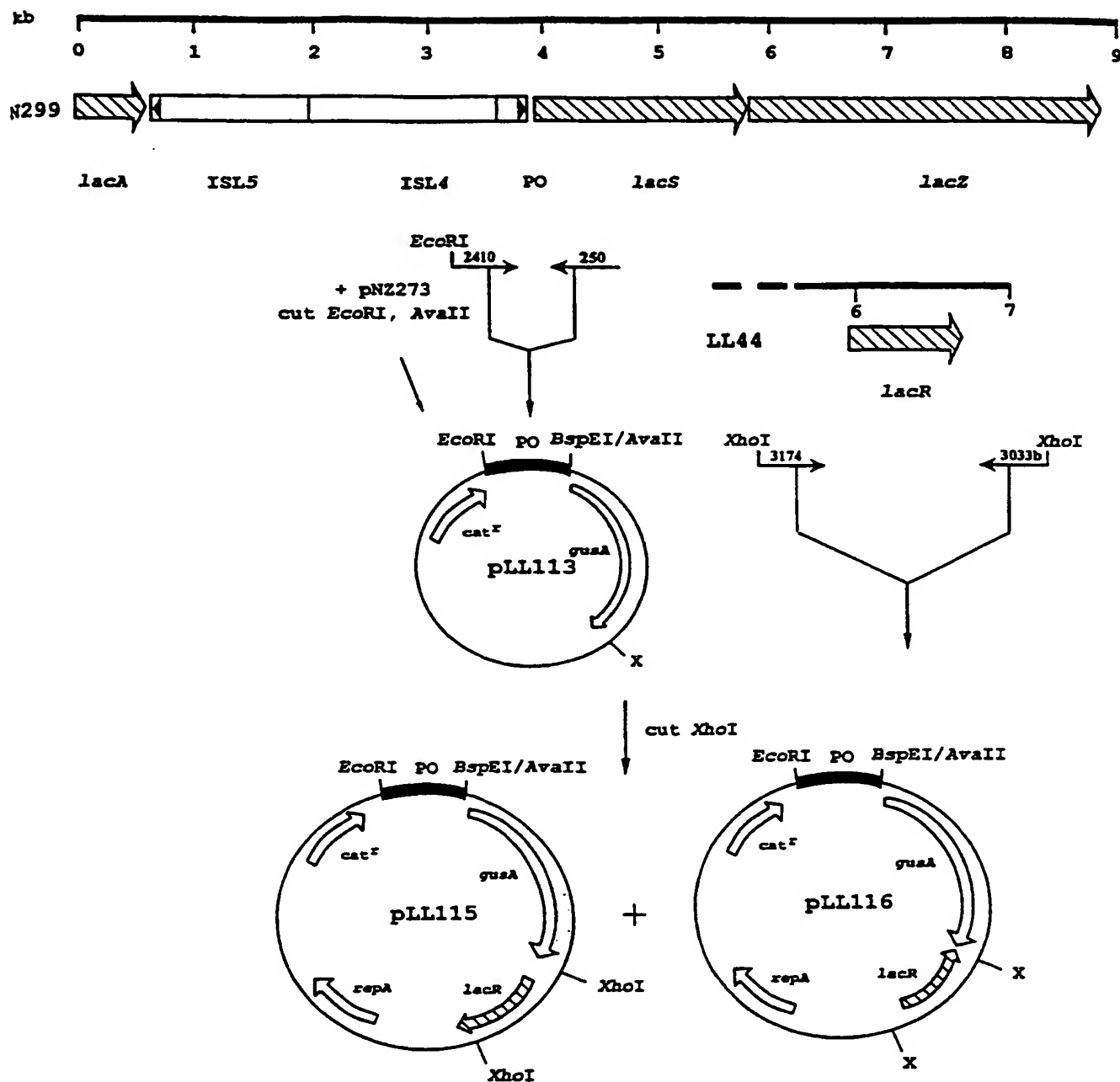
Physical map of the lactose operon of the different *L. delbrueckii* studied. Open arrows are for the *lac* operon genes and dashed arrow is for inactivated *lacR*. Boxes are for the different IS-elements, where the arrows heads are for the inverted repeats. \* same sequence as LL44 except an insertion in the 5' end of the *lacA* gene.



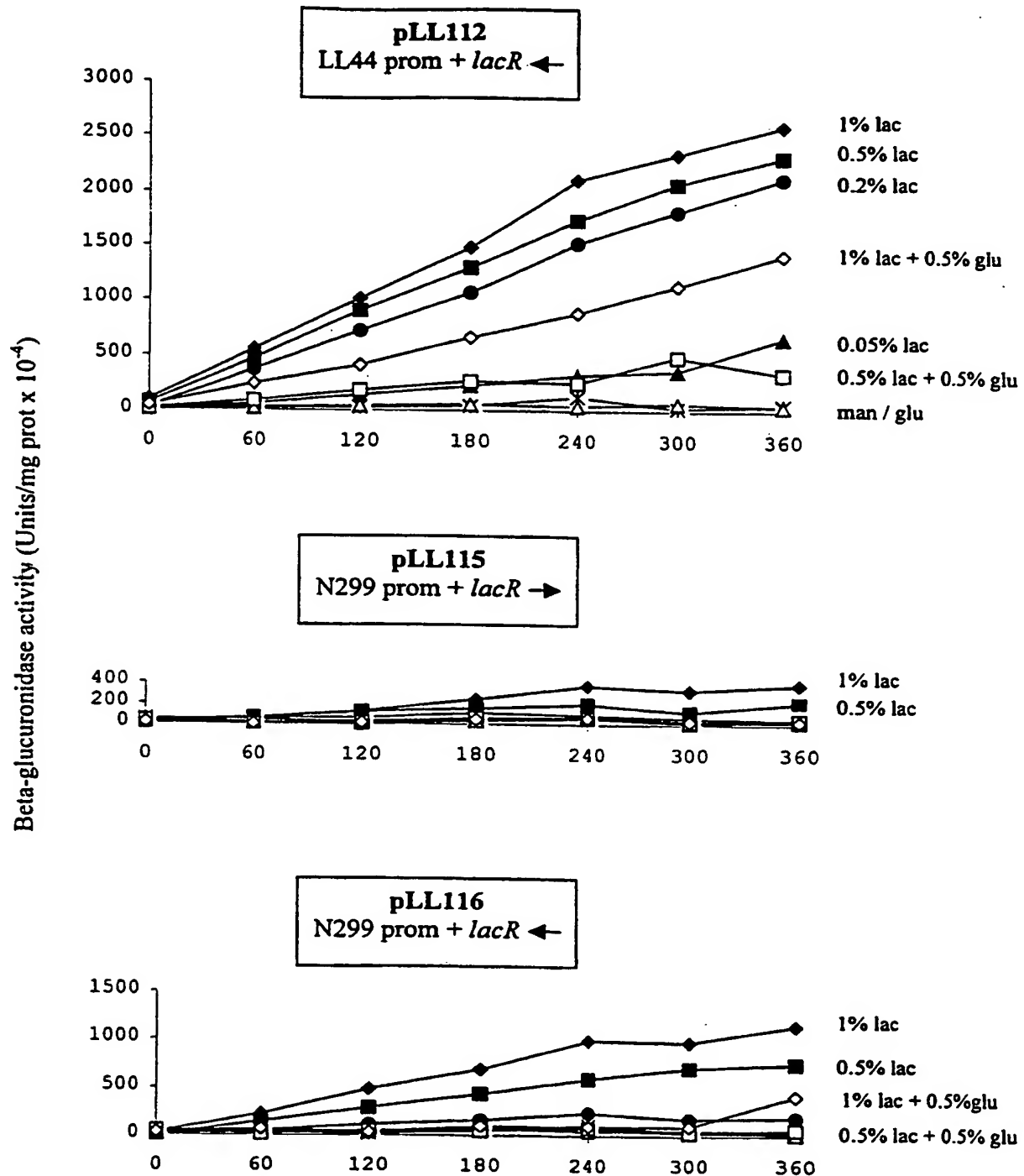
**Figure 6 :** Schematic representation of the construction of a pLL62. The dark box is for LL44 *lacR* gene and the white box is for the promoter region of the *lacI* gene of pET11c. Both were linked by PCR amplification using the SOEing method.



**Figure 7** : Schematic representation of the construction of pLL110 and pLL112. Dashed arrows are for the genes of the *L. delbrueckii* *lac* operon, and open arrows for plasmid genes. The dark box is for the promoter region cloned in front of the *gusA* gene. Plasmids are not drawn to scale. The simple arrows represent the primers used to amplify the cloned regions.

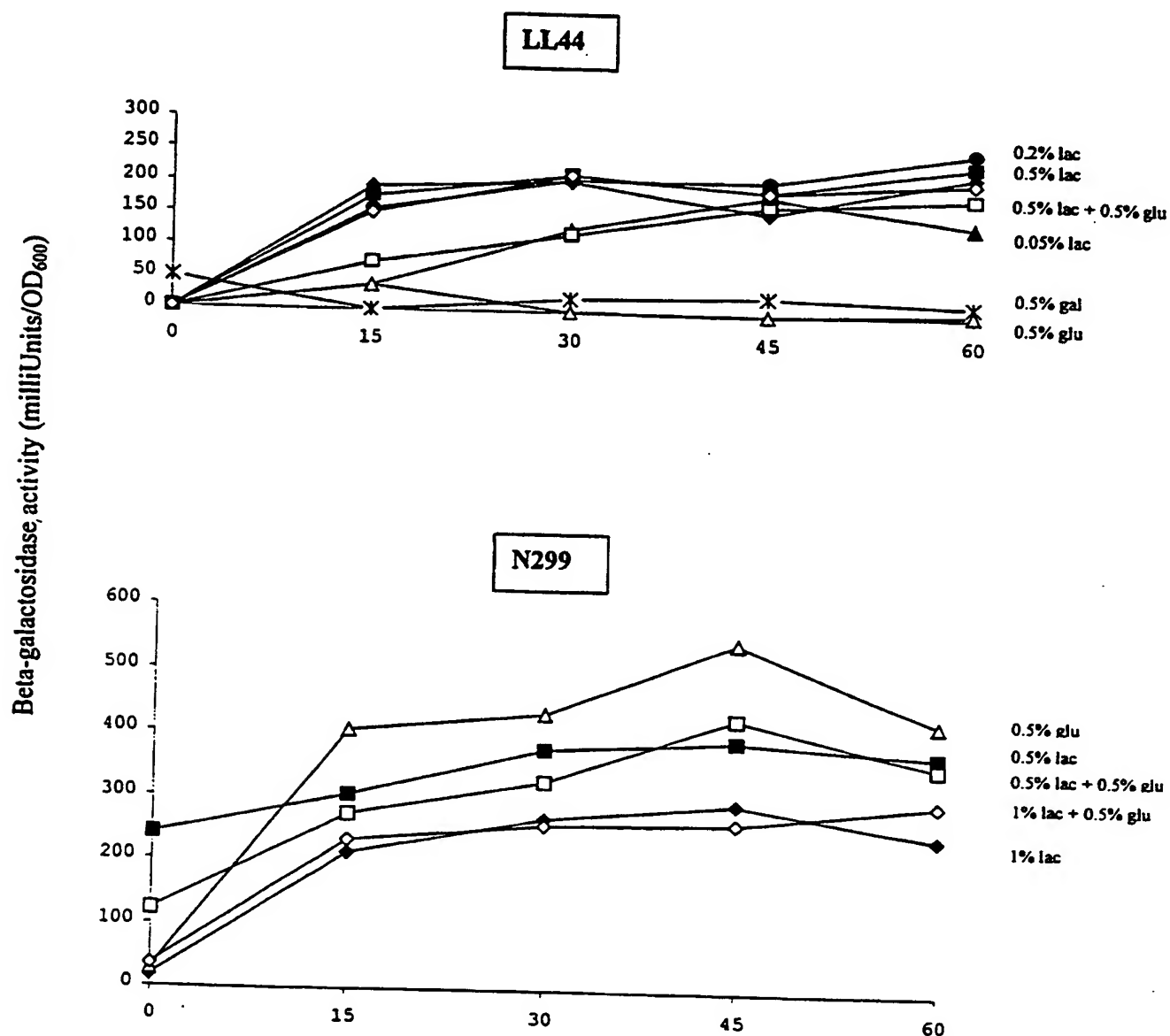


**Figure 8** : Schematic representation of the construction of pLL113, pLL115 and pLL116. Dashed arrows are for the genes of the *L. delbrueckii* *lac* operon, and open arrows for plasmid genes. The open box containing arrow heads represents the IS-elements. The dark box is for the promoter region cloned in front of the *gusA* gene. Plasmids are not drawn to scale. The simple arrows represent the primers used to amplify the cloned regions.



**Figure 9 :** Beta-glucuronidase activity (mean of three experiments) of *Lactococcus lactis* MG1363 containing different *Lactobacillus delbrueckii* *lac* promoter and the *lacR* gene of LL44. The *lacR* orientation compared to the *gusA* gene is represented by an arrow. The medium used was M17 containing : 0.5% mannose(\*), 0.05% lactose (▲), 0.2% lactose (●), 0.5% lactose (■), 1.0% lactose (◆), 0.5% glucose (△), 0.5% glucose + 0.5% lactose (□) and 0.5% glucose + 1.0% lactose (◇).





**Figure 10:** Beta-galactosidase activity (mean of three experiments) of *Lactobacillus delbrueckii* subsp. *lactis* LL44 and *L. delbrueckii* subsp. *bulgaricus* N299. The medium used was BHI-broth containing : 0.5% galactose (\*), 0.05% lactose ( $\Delta$ ), 0.2% lactose ( $\bullet$ ), 0.5% lactose ( $\blacksquare$ ), 1.0% lactose ( $\blacklozenge$ ), 0.5% glucose ( $\triangle$ ), 0.5% glucose + 0.5% lactose ( $\square$ ), and 0.5% glucose + 1.0% lactose ( $\diamond$ ). Strain N299 did not grow neither in galactose alone nor in 0.05% lactose and the experiment was not realised with these sugars.